International Journal of Plant & Soil Science

Volume 38, Issue 2, Page 71-78, 2026; Article no.IJPSS.146996
ISSN: 2320-7035

Effect of Brassinolide and Plant Spacing on Morphological and Yield Attributes of Lettuce

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2026/v38i25965

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/146996

Original Research Article

Received: 03/09/2025
Accepted: 07/11/2025
Published: 06/02/2026

Abstract

Lettuce (*Lactuca sativa* L.), a cool-season leafy vegetable of high nutritional and medicinal value, is globally recognised as a premier salad crop. The study aims to investigate the effect of brassinolide and plant spacing on morphological and yield attributes of Lettuce. The present study was conducted during the *Rabi* seasons of 2023–24 and 2024–25 at SHUATS, Prayagraj, to evaluate the effect of Brassinolide (BR) application and plant spacing on growth and yield attributes of lettuce. The experiment followed a factorial randomised block design comprising five Brassinolide levels (0, 4, 8, 12, and 16 ppm) and three plant spacings (45×30 cm, 45×45 cm, and 45×60 cm), with 15 treatment combinations replicated thrice. Results revealed that Brassinolide application at 12 ppm (B_4) significantly enhanced plant growth and productivity across both years and pooled data. This treatment recorded the highest plant height (30.21 cm), number of leaves per plant (27.39), leaf length (21.34 cm), width (20.12 cm), fresh (16.28 g) and dry (0.84 g) leaf weights, and maximum yield (251.72 q/ha). Plant spacing of 45×30 cm (S_1) also led to superior growth and yield,

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Cite as: *Prajwalitha, Saket Mishra, and Ali Sher. 2026. "Effect of Brassinolide and Plant Spacing on Morphological and Yield Attributes of Lettuce". International Journal of Plant & Soil Science 38 (2):71-78. https://doi.org/10.9734/ijpss/2026/v38i25965.*

with the highest values observed across most parameters. Interaction effects between BR levels and spacing were also significant. The findings suggest that the combined application of Brassinolide @ 12 ppm with a spacing of 45×30 cm optimises vegetative growth and yield of lettuce. This highlights the potential of BRs in enhancing physiological performance and productivity, especially under intensive cultivation systems. In conclusion, the application of Brassinolide improves the attributes to Brassinolide's role in promoting cell division, elongation, chlorophyll synthesis, and nutrient uptake, which collectively boost overall plant vigour and biomass accumulation.

Keywords: Brassinolide; herbaceous; lettuce; leafy vegetable; spacing; vitamins.

1. Introduction

Lettuce (*Lactuca sativa* L.), a member of the family Asteraceae, is widely regarded as the leading salad crop globally (Sahil et al., 2023). Lettuce is one of the most famous leafy vegetables worldwide, with lots of applications from food to other specific uses. There are different types of lettuce for consumers to choose from. Additionally, lettuce is an excellent source of bioactive compounds such as polyphenols, carotenoids, and chlorophyll with related health benefits (Shi *et al*., 2022; Meena *et al*., 2022). It is a leafy herbaceous plant characterised by the presence of a milky latex. Early in its growth cycle, lettuce forms a short stem, followed by a rosette of leaves that exhibit considerable variation in shape, colour, and texture across different cultivars (Ryder, 1979). This species is predominantly self-pollinated and possesses a diploid chromosome number of 2n = 18. Lettuce is well-suited to cool-season cultivation, with optimal growth occurring at daytime temperatures between 18°C and 25°C, and nighttime temperatures ranging from 10°C to 15°C (Ryder, 1998). The crop is believed to have originated in the Eastern Mediterranean region (Rubatzky & Yamaguchi, 1997). Lettuce is broadly classified into four primary horticultural types: Cos or Romaine (*Lactuca sativa var. longifolia*), Leaf lettuce (*Lactuca sativa var. crispa*), Head lettuce (*Lactuca sativa var. capitata*), and Stem lettuce (*Lactuca sativa var. asparagina*), among which head and leaf types are the most extensively cultivated worldwide.

Lettuce holds significant nutritional value due to its high mineral content and moderate levels of vitamins, making it a beneficial component of the human diet. It is particularly noted for its richness in fibre and water (Mou, 2009). A good source of pyridoxine, riboflavin and thiamine is in lettuce. It also offers some protein, carbs, dietary fibre and a negligible quantity of fat. Additionally, lettuce offers elements primarily present in the leaf, such as calcium, iron and copper. Most people eat lettuce raw in salads and sandwiches (Lal *et al*., 2024). Nutritionally, lettuce contains proteins, carbohydrates, and essential vitamins such as vitamin C and vitamin A. Per 100 grams of edible portion, lettuce comprises 93.4 g of moisture, 2.1 g of protein, 0.3 g of fat, 1.2 g of minerals, 0.5 g of fibre, and 2.5 g of carbohydrates. It also provides 310 mg of calcium, 80 mg of phosphorus, 2.6 mg of iron, 1650 I.U. of vitamin A, 0.09 mg of thiamine, 0.13 mg of riboflavin, and 10 mg of vitamin C. As a premium salad crop, lettuce is often served alongside vegetables such as tomatoes, carrots, and cucumbers. Additionally, Shi *et al.* (2022) highlighted the medicinal attributes of lettuce, noting its anodyne, sedative, diuretic, expectorant, and anti-rheumatic properties. Brassinosteroids (BRs) are plant-derived compounds that function as potent growth regulators, playing a vital role in modulating numerous physiological processes (Bishop *et al.* 2006). BRs are a group of polyhydroxylated steroidal phytohormones that are required for the development, growth, and productivity of plants. These hormones are involved in regulating the division, elongation, and differentiation of numerous cell types throughout the entire plant life cycle (Manghwar *et al*., 2022). These include promoting cell division and elongation, facilitating reproductive and vascular development, influencing leaf epinasty, regulating membrane polarization and proton pump activity, and modulating source–sink relationships (Sridhara *et al.,* 2021). Beyond their growth-promoting functions, BRs are also known for enhancing plant resilience under various stress conditions due to their strong antioxidant properties (Behnamnia *et al.* 2015). Under water stress, BRs contribute to elevated proline accumulation by inducing proline biosynthesis genes, thereby aiding in osmotic adjustment. Additionally, BR application significantly boosts carotenoid content during drought stress, which protects chlorophyll molecules from photo-oxidative damage, leading to improved chlorophyll retention and enhanced photosynthetic performance. Furthermore, exogenous application of BRs stimulates the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX), which play a critical role in scavenging reactive oxygen species (ROS) and mitigating oxidative damage (Behnamnia *et al.*, 2015). The study aims to investigate the effect of brassinolide and plant spacing on morphological and yield attributes of Lettuce.

2. Materials and Methods

The present investigation was meticulously carried out at the Horticultural Research Farm of the Department of Horticulture, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj, Uttar Pradesh, during the Rabi seasons of 2023-24 and 2024-25. The field is strategically situated approximately 5 kilometres from Prayagraj City, along the Prayagraj–Rewa National Highway, offering convenient accessibility. The experimental design encompassed the time period from November to February of 2023-2024 and November to February of 2024-2025. It followed a two factor factorial randomized block design, consisting of two factors of Plant spacing and Brassinolide application with three and five levels respectively comprising of fifteen treatment combinations and three replications. The treatments comprised Factor (I) - B_1 (Control); B_2 (Brassinolide 0.01% SP @ 4 ppm); B_3 (Brassinolide 0.01% SP @ 8 ppm); B_4 (Brassinolide 0.01% SP @ 12 ppm); B_5 (Brassinolide 0.01% SP @ 16 ppm). Factor (II)- S_1 (45 cm × 30 cm); S_2 (45 cm × 45 cm) and S_3 (45 cm × 60 cm). Characters like Plant height (cm); Number of leaves per plant; Leaf length and width (cm); Fresh and Dry weight of leaf (g), and Yield per hectare (kg/ha). Analysis of Variance was worked out using Panse and Sukhatme (2000).

3. Results

3.1 Plant Height

The application of Brassinolide and different plant spacings significantly influenced the plant height of lettuce during both 2022–23 and 2023–24, as well as on a pooled basis. Among the treatments, B_4 [Brassinolide 0.01% SP @ 12 ppm] recorded the highest plant height with values of 29.25 cm (2023–24), 31.18 cm (2024–25), and 30.21 cm (pooled), followed closely by B_3 [Brassinolide 0.01% SP @ 8 ppm] with 28.74 cm, 30.45 cm, and 29.59 cm, respectively. The lowest height was observed in the control (B_1), which recorded 22.13 cm, 23.44 cm, and 22.79 cm. B_3 was statistically at par with B_4 across both years and the pooled data. Regarding plant spacing, S_1 [45 cm × 30 cm] produced the tallest plants with heights of 27.01 cm (2023–24), 28.74 cm (2024–25), and 27.88 cm (pooled), followed by S_2 [45 cm × 45 cm] with values of 26.24 cm, 27.79 cm, and 27.02 cm, respectively. The shortest plants were observed in S_3 [45 cm × 60 cm], which showed heights of 25.08 cm, 26.56 cm, and 25.82 cm. The effect of S_2 was statistically at par with S_1 in both years and also with S_3 in 2024–25. A significant interaction was also observed between Brassinolide application and plant spacing.

3.2 Number of Leaves Per Plant

The influence of Brassinolide application on the number of leaves per plant in lettuce revealed statistically significant variations across both years (2022–23 and 2023–24) and in the pooled data. The highest number of leaves per plant was observed with B_4 [Brassinolide 0.01% SP @ 12 ppm], recording 26.73, 28.05, and 27.39 leaves in 2023–24, 2024–25, and pooled data, respectively. This was followed by B_3 [Brassinolide 0.01% SP @ 8 ppm], which showed 26.46, 27.71, and 27.09 leaves per plant across the respective periods. The control (B_1) consistently recorded the lowest number of leaves, with values of 20.33, 20.86, and 20.60. Notably, the performance of B_3 was statistically at par with B4 across both years and pooled results. In terms of plant spacing, significant differences were also recorded, with the S1 spacing (45×30 cm) yielding the highest number of leaves per plant—24.90, 26.02, and 25.46 during 2023–24, 2024–25, and pooled data, respectively—closely followed by S_2 (45×45 cm), which recorded 24.50, 25.49, and 24.99. The lowest number was observed in S_3 (45×60 cm), with 23.41, 24.33, and 23.87 leaves. The performance of S_2 was statistically at par with S_1 across all datasets.

3.3 Leaf Length and Width (cm)

The pooled mean data revealed that Brassinolide application and plant spacing significantly influenced the leaf length and width of lettuce. Among the treatments, application of B_4 [Brassinolide 0.01% SP @ 12 ppm] recorded the highest pooled leaf length (21.34 cm) and width (20.12 cm), followed by B_3 [8 ppm], which showed statistically at par values of 20.59 cm and 20.01 cm, respectively. The control (B_1) consistently exhibited the lowest pooled leaf length (15.51 cm) and width (16.32 cm). Regarding plant spacing, S_1 [45×30 cm] resulted in maximum pooled leaf length (19.05 cm) and width (19.49 cm), followed by S_2 [45×45 cm] with 18.69 cm and 18.67 cm, respectively, both being statistically at par. The widest spacing S_3 [45×60 cm] recorded the lowest pooled values (17.90 cm length and 17.80 cm width).

3.4 Fresh and Dry Weight of Leaf (g)

The application of Brassinolide significantly influenced both fresh and dry weights of lettuce leaves across 2022–23, 2023–24, and pooled data. Among treatments, B_4 (Brassinolide @ 12 ppm) consistently recorded the highest fresh (16.28 g) and dry (0.84 g) leaf weights, followed by B_3 (8 ppm), while B_1 (Control) showed the lowest values (13.35 g fresh and 0.61 g dry weight). Notably, B_3 was statistically at par with B_4, and B_5 (16 ppm) performed similarly to the control. Regarding plant spacing, S_1 (45×30 cm) produced the highest fresh (15.41 g) and dry (0.77 g) leaf weights, statistically at par with S_2 (45×45 cm), while S_3 (45×60 cm) yielded the lowest (14.46 g fresh and 0.69 g dry weight).

3.5 Yield Per Hectare (kg/ha)

The application of Brassinolide and variation in plant spacing significantly influenced the yield per hectare (q/ha) of lettuce during both 2023–24 and 2024–25 as well as in the pooled data. Among the treatments, application of B_4 [Brassinolide 0.01% SP @ 12 ppm] recorded the highest yield per hectare with 238.21 q/ha in 2023–24, 265.24 q/ha in 2024–25, and 251.72 q/ha in pooled data, followed by B_3 [Brassinolide 0.01% SP @ 8 ppm] with 221.10, 240.95, and 231.02 q/ha respectively, while the lowest was under B_1 [Control], which recorded 148.11, 156.18, and 152.14 q/ha. Among spacing levels, S_1 [45×30 cm] produced the maximum yield per hectare (281.39, 304.98, and 293.18 q/ha), followed by S_2 [45×45 cm] (181.65, 196.15, and 188.90 q/ha), whereas the minimum yield was observed with S_3 [45×60 cm] (124.66, 133.67, and 129.17 q/ha).

4. Discussion

The findings of this study highlight the pronounced influence of Brassinolide application and plant spacing on the growth and yield attributes of lettuce. Application of Brassinolide, particularly at 12 ppm (B_4), significantly enhanced plant height, number of leaves, leaf dimensions, biomass, and ultimately yield per hectare. The highest plant height (30.21 cm pooled) and number of leaves per plant (27.39 pooled) were recorded under B_4, closely followed

Table 1. Effect of Brassinolide and Plant spacing on plant height (cm) of Lettuce

Plant height (cm)			
Treatments	**2023-24**	**2024-25**	**Pooled**
Brassinolide (B)			
B_1: Control	22.13	23.44	22.79
B_2: Brassinolide 0.01% SP @ 4 ppm	26.24	27.79	27.02
B_3: Brassinolide 0.01% SP @ 8 ppm	28.74	30.45	29.59
B_4: Brassinolide 0.01% SP @ 12 ppm	29.25	31.18	30.21
B_5: Brassinolide 0.01% SP @ 16 ppm	24.20	25.63	24.91
F-Test	S	S	S
S.E. (m) (±)	0.46	0.55	0.36
CD at 0.05 %	1.35	1.61	1.02
Plant Spacing (S)			
S_1: 45cm×30cm	27.01	28.74	27.88
S_2: 45cm×45cm	26.24	27.79	27.02
S_3: 45cm×60cm	25.08	26.56	25.82
F-Test	S	S	S
S.E. (m) (±)	0.36	0.43	0.28
CD at 0.05 %	1.04	1.25	0.79

Table 2. Effect of Brassinolide and Plant spacing on number of leaves per plant of lettuce

Number of leaves per plant			
Treatments	**2023-24**	**2024-25**	**Pooled**
Brassinolide (B)			
B_1: Control	20.33	20.86	20.60
B_2: Brassinolide 0.01% SP @ 4 ppm	24.84	25.91	25.38
B_3: Brassinolide 0.01% SP @ 8 ppm	26.46	27.71	27.09
B_4: Brassinolide 0.01% SP @ 12 ppm	26.73	28.05	27.39
B_5: Brassinolide 0.01% SP @ 16 ppm	22.96	23.87	23.42
F-Test	S	S	S
S.E. (m) (±)	0.42	0.49	0.32
CD at 0.05 %	1.22	1.42	0.91
Plant Spacing (S)			
S_1: 45cm×30cm	24.90	26.02	25.46
S_2: 45cm×45cm	24.50	25.49	24.99
S_3: 45cm×60cm	23.41	24.33	23.87
F-Test	S	S	S
S.E. (m) (±)	0.33	0.38	0.25
CD at 0.05 %	0.95	1.10	0.71

Table 3. Effect of Brassinolide and Plant spacing on leaf length and width (cm) of Lettuce

Treatments	**Leaf length (cm)**			**Leaf width (cm)**		
	2023-24	**2024-25**	**Poole**	**2023-24**	**2024-25**	**Pooled**
Brassinolide (B)						
B_1: Control	15.27	15.75	15.51	15.98	16.66	16.32
B_2: Brassinolide 0.01% SP @ 4 ppm	18.41	18.99	18.70	18.29	18.99	18.64
B_3: Brassinolide 0.01% SP @ 8 ppm	20.28	20.91	20.59	19.61	20.42	20.01
B_4: Brassinolide 0.01% SP @ 12 ppm	20.94	21.74	21.34	19.81	20.43	20.12
B_5: Brassinolide 0.01% SP @ 16 ppm	16.88	17.39	17.12	17.08	17.80	17.44
F-Test	S	S	S	S	S	S
S.E. (m) (±)	0.42	0.46	0.31	0.41	0.38	0.28
CD at 0.05 %	1.22	1.32	0.88	1.18	1.10	0.79
Plant Spacing (S)						
S_1: 45cm×30cm	19.15	19.83	19.49	18.70	19.35	19.02
S_2: 45cm×45cm	18.38	18.95	18.67	18.21	18.96	18.59
S_3: 45cm×60cm	17.53	18.08	17.80	17.55	18.26	17.91
F-Test	S	S	S	S	S	S
S.E. (m) (±)	0.33	0.35	0.24	0.32	0.29	0.22
CD at 0.05 %	0.95	1.02	0.68	0.91	0.85	0.61

by B_3 (8 ppm), suggesting that Brassinolide promotes vegetative growth, likely due to its role in enhancing cell elongation and division. Likewise, leaf length and width were greatest under B_4 (21.34 cm and 20.12 cm pooled), further reflecting the hormone's positive influence on leaf development. Yield parameters such as fresh and dry weight of leaves also showed maximum values under B_4, with pooled fresh and dry weights of 16.28 g and 0.84 g, respectively. This cumulative increase in vegetative traits translated into significantly higher yield per hectare under B_4 (251.72 q/ha pooled), followed by B_3 (231.02 q/ha), with the control treatment consistently yielding the lowest across all parameters. Similarly, plant spacing played a crucial role, where closer spacing (S_1: 45×30 cm) produced the tallest plants, most leaves, and the highest biomass and yield per hectare (293.18 q/ha pooled). This may be attributed to optimal resource use per unit area despite potential competition. Wider spacing (S_3: 45×60 cm), in contrast, resulted in the lowest values for all growth and yield traits, indicating suboptimal utilization of space and inputs.

The application of B_4 [Brassinolide 0.01% SP @ 12 ppm] significantly influenced various growth parameters of lettuce, including plant height, number of leaves, leaf size, and biomass

Table 4. Effect of Brassinolide and Plant spacing on fresh and dry weight of leaf (g) of Lettuce

Treatments	Fresh weight of leaf (g)			Dry weight of leaf (g)		
	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled
Brassinolide (B)						
B_1: Control	13.15	13.55	13.35	0.60	0.62	0.61
B_2: Brassinolide 0.01% SP @ 4 ppm	15.00	15.29	15.15	0.73	0.75	0.74
B_3: Brassinolide 0.01% SP @ 8 ppm	15.62	16.31	15.97	0.79	0.83	0.81
B_4: Brassinolide 0.01% SP @ 12 ppm	15.82	16.75	16.28	0.82	0.86	0.84
B_5: Brassinolide 0.01% SP @ 16 ppm	14.08	14.56	14.32	0.66	0.69	0.68
F-Test	S	S	S	S	S	S
S.E. (m) (±)	0.34	0.36	0.25	0.03	0.03	0.02
CD at 0.05 %	0.99	1.04	0.70	0.08	0.09	0.06
Plant Spacing (S)						
S_1: 45cm×30cm	15.13	15.70	15.41	0.76	0.79	0.77
S_2: 45cm×45cm	14.88	15.45	15.16	0.73	0.76	0.75
S_3: 45cm×60cm	14.19	14.72	14.46	0.68	0.70	0.69
F-Test	S	S	S	S	S	S
S.E. (m) (±)	0.27	0.28	0.19	0.02	0.02	0.02
CD at 0.05 %	0.77	0.81	0.54	0.06	0.07	0.04

Table 5. Effect of Brassinolide and Plant spacing on yield per plot (kg) and yield per hectare (q/ha) of Lettuce

Treatments	Yield per hectare (q/ha)		
	2023-24	2024-25	Pooled
B_1: Control	148.11	156.18	152.14
B_2: Brassinolide 0.01% SP @ 4 ppm	197.89	209.41	203.65
B_3: Brassinolide 0.01% SP @ 8 ppm	221.10	240.95	231.02
B_4: Brassinolide 0.01% SP @ 12 ppm	238.21	265.24	251.72
B_5: Brassinolide 0.01% SP @ 16 ppm	174.19	186.23	180.21
F-Test	S	S	S
S.E. (m) (±)	7.57	4.57	4.42
CD at 0.05 %	21.93	13.24	12.53
S_1: 45cm×30cm	281.39	304.98	293.18
S_2: 45cm×45cm	181.65	196.15	188.90
S_3: 45cm×60cm	124.66	133.67	129.17
F-Test	S	S	S
S.E. (m) (±)	5.86	3.54	3.42
CD at 0.05 %	16.99	10.25	9.70

accumulation. The highest plant height was observed under B_4, likely due to the role of brassinolide in promoting cell division and elongation. This phytohormone binds to specific receptors, triggering genetic and physiological responses that facilitate growth by stimulating enzyme and protein synthesis involved in cell wall loosening, allowing for enhanced cell expansion (Manghwar *et al.*, 2022). Additionally, brassinolide boosts photosynthetic efficiency and nutrient uptake, enabling the plant to direct more energy toward vertical growth, thereby increasing height (Haubrick & Assmann, 2006). Similar increases in plant height under brassinolide application were reported by Benavides *et al.* (2023) and Doležalová *et al.* (2016) in lettuce. Moreover, B_4 also recorded the highest number of leaves per plant, which can be attributed to increased meristematic activity and enhanced energy availability due to improved chlorophyll synthesis and photosynthesis (Wang & Irving, 2011). This aligns with findings by Serna *et al.* (2012) and Benavides *et al.* (2023), who also observed enhanced leaf proliferation in lettuce under BR application. Leaf length and width were also maximized under B_4, likely due to brassinolide-induced stimulation of cell elongation and expansion. This effect is regulated through the modulation of genes involved in cell wall remodelling and leaf morphogenesis (Zhang *et al.*, 2009; Siddiqui *et al.*, 2018) and is influenced by environmental interactions such as light and temperature (Nakaya *et al.*, 2002). Enhanced leaf dimensions under BR application were similarly reported in Brassica by Naveen *et al.* (2021) and in tobacco by Zhang *et al.* (2021). Furthermore, fresh and dry weight of leaves also peaked under B_4. The increased fresh weight reflects improved water and nutrient uptake and vigorous metabolic activity, while the elevated dry weight indicates higher structural biomass accumulation, including cellulose and stored reserves like starch and sugars (Manghwar *et al.*, 2022; Kumari & Hemantaranjan, 2018).

5. Conclusion

The application of Brassinolide 0.01% SP @ 12 ppm (B_4) significantly enhanced plant height, number of leaves, leaf size, and both fresh and dry leaf weight in lettuce. These improvements are attributed to Brassinolide's role in promoting cell division, elongation, chlorophyll synthesis, and nutrient uptake, which collectively boost overall plant vigour and biomass accumulation. The hormone also aids in better stress tolerance and metabolic efficiency. Thus, B_4 proves to be an effective treatment for maximising vegetative growth and yield potential in lettuce, highlighting its value as a growth-promoting regulator in commercial leafy vegetable cultivation.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Acknowledgement

The author expresses sincere gratitude to the Head of the Department of Horticulture SHUATS, Prayagraj for their invaluable support and assistance.

Competing Interests

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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